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Letter to the Editor

Governance of Science at the National Cancer Institute: Perceptions and Opportunities in Oncogene Research[1]

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History of Funding

Pasteur said that there is no such thing as basic research and applied research, only basic research and the application of the results of basic research. Because of the multifaceted biological revolution, the time between discovery and application is shortening, and molecular biology is moving close to the bedside. The exciting tempo in cancer research provides a test for science management. The test is simply this. Allow maximum flexibility for outstanding scientists and make sure that connections are made as early as possible between clinicians and laboratory scientists. Nowhere will this capacity to connect be tested more than in the area of oncogene research, where basic knowledge in biology immediately conjures up images of "What if?" As a major supporter of research in this area, the NCI[3] has made a commitment to make these connections, if possible, as soon as possible. Some aspects, opportunities, and commitments are the subject of this essay.

The success of oncogene research can be directly traced to support of virus cancer research which began in 1964 and expanded significantly in the early 1970s. In 1970, $21 million was spent. In 1975, the total had quadrupled. By 1976, the total amount of combined extramural and intramural research for all funding mechanisms rose above $100 million per year, and it remained at that level until 1981. In a previous report (6), a commentary was given on the change in the arrangement of the Viral Oncology/Biological Carcinogenesis program from one supported by contracts to a grant program. This change paralleled the shift from the search for cancer viruses to research in the molecular genetics of rapidly transforming retroviruses that led into the area of oncogene research. Whereas prior to 1981 all oncogene research was associated with virus cancer research, after this time there began to be a significant amount spent on cellular transforming genes, which were technically not associated with viral research. During the top 5 years of virus-associated cancer research, 12.5% of the entire NCI's budget was expended in this area. During the last 2 years, the proportionate amount of NCI's total budget spent on virus cancer research was about 9%, or $90 million. It is noteworthy that the total amount spent on cancer virus research in the last 11 years just exceeded $1 billion.

Within the past few years, research on oncogenes has become clearly identified in its own right so that funding allocations could be more precisely defined. In 1983, the total amount expended on pure oncogene research was $36 million, which represented 3.7% of the total NCI budget. The proportion of cancer research defined as strictly oncogene research is rising and is expected to continue to go up by 8.4% in 1984. Our research budget projections indicate a similar growth into 1985.

Contrary to some views, viral oncology research is therefore an active and healthy special initiative of the Cancer Program. Although not the subject of this report, the recent reactivation of interest in human retroviruses (10) has reawakened interest in expanding the research effort on retroviruses as a cause of human disease, now that more sophisticated tools are available.

Two years ago, NCI effected a major shift of emphasis directly to oncogenes, so that a major focus of the NCI's largest basic research contract at the Frederick Cancer Research Facility has become oncogene research. The newest research emphasis is the planned acquisition of the first supercomputer dedicated to biomedicine (16). Studies in comparative molecular dynamics and modeling of oncogene products, as well as oncogene sequence analyses, will be significantly enhanced by advanced computing.

Development of the Oncogene Concept

It may be appropriate to define the historical perspective for the recent findings. The earliest insights, starting about 12 years ago, have come from the studies of a class of animal viruses which could cause leukemias and solid cancers in animals (9, 14, 24). These viruses belonged to a group called "retroviruses" because their genetic information existed in the form of RNA, rather than in the usual form of DNA. These viruses had the potential to add unwanted genetic information to normal cells. A second important characteristic of retroviruses was that they possess a molecular switching device, capable of turning on various genes (2). If such a switch or promoter were to be placed near an inactive gene, it can activate the gene to function in an abnormal way.

Some of the most fascinating retroviruses were those which caused leukemias or solid tumors very rapidly in test animals. These rapid tumor-inducing viruses were unusual because most of them were defective in that they lacked one or more genes needed for their own multiplication. Evidently, after losing their own genes, retroviruses picked up and carried about some specific cellular genes. It was the cellular gene that the virus had picked up rather than one of its own genes that caused the cancer. This implied that the potential for cancer may be encoded in part in the genes of animals, including humans. It is these cellular genes, the action of which could result in malignant behavior, that have been named oncogenes. It was clear that a number of rapidly transforming retroviruses could carry about such cellular oncogenes. Up to now, about 20 such genes have been isolated. Although the cell has tens of thousands of poten-

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tially active genes, it appears that the cell has only several dozen oncogenes.

Another parallel approach which developed within the last 5 years has been based on the idea that tumor cells themselves could possess turned-on oncogenes. Because most human tumors are not known to be associated with viruses, it was thought that, if the DNA of the tumor cell itself were transferred to another normal cell, the effect of the tumor cell DNA would be dominant and transform the normal cell to a malignant one. The process of transferring naked DNA from tumor cell to recipient cell, called "transfection," was found to cause cell transformation (22). Very regularly, scientists could take DNA from most types of tumors and, with a reproducible rate of success, were able to transform apparently normal cells in the laboratory into malignant cells. A particularly important finding was that many of those cellular oncogenes, derived directly from tumors, were essentially the same as several of the oncogenes that were first found to be carried around by the rapidly transforming retroviruses described earlier.

How Does an Oncogene Act?

The next important question was, "If the cell has the information for 22 oncogenes, why isn't the cell abnormal?" Because all genes act through their protein products, one must examine the status of the oncogene protein itself. Two general alternatives are possible: (*a*) that an oncogene functions excessively; or (*b*) that the oncogene itself has become abnormal in some way and that the abnormal protein product results in cancer.

The first possibility of an overproduction of a normal oncogene product has been clearly documented (5). We now know that there are retroviruses without oncogenes which can cause tumors only after a long waiting period. When the DNA of these tumors is examined, the infecting virus or a part of it is found in tumor cell DNA. The critical part of the virus is the powerful "on" switch known as a promoter. This part of the virus has integrated next to a known cellular oncogene and, because of the strength of the promoter, the oncogene became hyperactive. Such indirect activation of cellular oncogenes has been named the "promoter insertion model" (13).

The second form of oncogene activation is a specific mutational change which alters the DNA in an irreversible way. Experiments of this kind with chemical carcinogens have been able to precisely tie in the nature of the chemical action exerted by the carcinogen on the DNA and the resulting mutational mistake in the DNA of the oncogene. Such an activated oncogene can then be isolated, transfected, and shown to act as a transforming oncogene. The fact that most known carcinogens have an effect on DNA, which can cause misreading of the genetic code, implies that the way chemical carcinogens act may be through the specific activation of previously normal cellular oncogenes.

Studies in chemical carcinogenesis indicate that cancer develops by a multistep process (26). Therefore, the question arose whether oncogenes also act sequentially, in concert with each other in the laboratory. To test this, normal cells have been taken directly from the animal and transfected with DNA containing a known active oncogene. The cells did not become malignant. However, if a second oncogene was transfected into the same cells, then a full-blown transformation was seen (15, 20). In other instances, some rapidly transforming retroviruses already carry 2 different oncogenes. Thus, one can identify cancer as a multistep process under laboratory conditions, as well as in epidemiological studies.

Normal Function of Oncogenes in Cells

A very basic question is, what are these oncogenes found in normal cells all about? Are they merely the enemy within (1), or do they have important natural functions in cell development?

One of the features of any gene is its rate of evolution. A general rule is that genes which are important for basic functions will evolve slowly and remain very similar in all forms of life. When human oncogenes were examined, they were found to be extremely well conserved so that similar genes were found even in fruit flies and yeasts. Because such genes are easier to analyze in lower organisms, it became clear that oncogene-like genes in such organisms act as important growth control signals.

On the human level, we now know that the protein products of several known oncogenes are involved in growth control. The first of these was the product of a monkey-derived oncogene called *sis* (27). A computer was used to search for the sequence of the *sis* oncogene among many other genes which have been sequenced. An almost perfect match was found with the sequence for the normal platelet-derived growth factor gene (8). Platelet-derived growth factor is a small protein found in blood platelets which is very much involved with wound healing and growth repair processes. Another computer search found that the sequence of an avian oncogene, called *erbB*, was very analogous to a gene coding for the cellular receptor required to bind a small molecule called the epithelial growth factor (7). Thus, based on these 2 examples, it seems that cellular oncogenes have a clearly defined, normal function in some cells at some time.

Ability of Oncogenes to Move Around in Cells

Today the specific chromosomes on which most of the known oncogenes are located have been identified. Highly specific chromosomal changes occur in all tumors of certain types, and it was therefore reasonable to suspect that oncogenes may be involved in these changes. In a lymph cell tumor of children, called Burkitt's lymphoma, scientists have known for some time that small pieces of 2 different chromosomes break off. These pieces are then exchanged reciprocally, so that in the final form the chromosomes end up with wrong pieces reglued to their main parts. These abnormal chromosomes are now known to be directly involved in the cancer process. One of the broken off fragments has a known oncogene, and the major piece of the other chromosome to which this oncogene becomes fused is involved in the control of the synthesis of antibodies. Thus, an abnormal situation results in that incorrect reading and expression of an oncogene occurs because of the action of an incorrect control element. A number of other such instances abound or are suspected in other forms of cancer. Sometimes 2 oncogenes are mutually exchanged between different chromosomes. This area of study is likely to prove useful in developing better diagnostic approaches in cancers with chromosome changes that are more subtle.

One of the observations at the level of chromosomes has been the appearance of abnormal regions within a chromosome which do not stain normally, or of many tiny pieces of chromosomes broken free from the normal structures. This was first seen in certain cancers. We now know that this is due to a vast increase,

or an amplification, in the number of genes coding for a particular function. Gene amplification is also a feature of oncogenes. In the course of tumor growth and progression, a number of different cancers have shown an amplification of several oncogenes. Although this is generally a late effect, it can be perceived as making the cell more aggressive and growth independent. Future approaches may well include strategies to interfere with gene amplification.

Possible Opportunities in Diagnosis, Prevention, and Treatment of Cancer

Although, at present, most of these findings exist at the research level, modern biotechnology can overcome the technical barriers to bring these advances to the patient's bedside. Recent events in the use of recombinant DNA technology have shown that carrier states of several serious genetic diseases, such as Huntington's disease, can be identified with certainty at the molecular level by identifying specific changes in the genes (12). In cancer, the participation and role of built-in genetic changes, which may predispose at least to some cancers, is well known. For example, 3 childhood tumors have a defined genetic basis. One of these, retinoblastoma, a tumor of the eye, is known to be associated with chromosomal abnormalities, some of which are manifest as a loss of a part of a chromosome (17). Specific nucleic acid probes could be used to identify the potential carriers of this disease. In Wilm's tumor, a childhood tumor of the kidney, a small piece of chromosome 2 is missing from an area adjacent to the oncogene known to be present on the same chromosome (18). In neuroblastomas, deletions of bands on chromosome 1 have also been identified (4). Again, precise molecular probes cannot only readily detect the specific chromosome abnormalities in the child with a tumor but may also make it possible to identify the carrier state in the parents of the Wilm's tumor patient.

An expansion of these concepts to other conditions which are known to be strongly predisposing to cancer is under way. For example, in the hereditary Gardner's syndrome, in which multiple polyps are observed in the colon, these people often develop colon cancer. Colon cancer cells generally express an activated oncogene (23). Although the normal cells from unaffected parts of the body of these patients have not displayed activated transforming genes, more studies would be needed in cells of the lining of the colon in which malignant changes first appear. Based on the multistep nature of the cancer process, it may be that other oncogenes, which are not detectable in transfection assays, may already be in an activated or a permuted state. The approach that could make this possible is the use of restriction fragment-length polymorphism analysis (3). The presence of altered gene sequences, which could arise by a number of mechanisms, such as deletions, rearrangements, amplifications, etc., can be identified. The specific examination for mutational changes in known oncogenes with restriction endonucleases would give a more detailed picture.

The above instances clearly show that specific genetic characteristics render certain populations at increased risk for cancer. However, it is also well known that the most prevalent and potentially lethal carcinogen exposure in humans, tobacco smoke, will not affect all individuals in the same way. Many people know very old, heavy smokers who continue to be healthy. On a statistical basis, the heaviest smokers, who are at the highest risk of developing lung cancer, have a 10 to 20% chance of actually getting the disease. To be able to identify those tobacco users who would be more susceptible to early tumor induction would be of immense benefit. The cancer cells of patients with lung tumors also have an activated oncogene (23). In contrast, the normal lung cells from the same patient will not have an activated form of the gene, implying that the change occurred as part of the tumor induction, and was presumably initiated as mutational DNA damage by the carcinogen in the tobacco smoke. In all of these cases, the underlying principle is that a genetic change, which can be identified molecularly, would specify a predisposition to cancer. The knowledge that multiple oncogenes can act in concert and the availability of known oncogene probes as well as their surrounding control regions may facilitate identification of the at-risk individuals and allow the precise testing of approaches to prevent cancer in defined populations.

Attack on the Oncogene Product

One of the exciting areas has been the identification of the protein products of the activated oncogenes. Because activation is associated with a change in the DNA, the resulting protein will also have different structure as well as properties. The new and powerful monoclonal antibody technology can identify changes on the surface of a protein with great precision. Thus, one can theoretically develop antibodies to a site on the activated oncogene protein which can differentiate between the product of the activated oncogene and the normal nonactivated cellular oncogene.

Monoclonal antibodies can also be armed with radioactive tracer molecules, to zero in on small areas of tumor cells undetectable by any other diagnostic method. Alternatively, monoclonal antibodies could serve as carriers for drugs and toxins to deliver treatment directly to the tumor cell. The specific recognition of a cell, which has an abnormal oncogene protein, by such an armed antibody would be valuable in both pathological diagnosis and treatment. We already have monoclonal antibodies to tumor-specific surface proteins of many cancers. These antibodies are already being used experimentally for the diagnosis of distant tumor spread (21). A potential limitation of this approach is that not all tumor cells will make enough protein antigen for identification. Furthermore, tumor cells can turn off proteins if these are not needed for the maintenance of the malignant state.

A possible strategy may be to attack the protein product of the activated oncogene itself by monoclonal antibodies. This should work because the oncogene protein is the key which makes the cell malignant. One of the problems, however, is that only some of the oncogene products are present at the cell surface. The activated proteins of other oncogenes lie underneath the cell membrane or even in the nucleus of the cell. New technologies arising from concepts of receptor-mediated endocytosis (11) are now being explored at the NCI to bring armed antibodies or their fragments to the inside of the cancer cell (19).

Alternatively, graphic displays of molecular models of normal or activated oncogene products could be visualized with advanced computer technology. Selective theoretical substitutions of amino acids could give insights into the molecular dynamics of the normal *versus* the abnormal states. Identification of active sites could lead to designs of possible inhibitors. Exciting work in the area of structural changes and enzyme inhibitors have

been initiated in the case of dihydrofolate reductase (25). The access of cancer researchers to a supercomputer, which is needed to effect these studies, should be of great benefit.

Another problem that has been observed is that oncogene amplification is proportional to the degree of malignant behavior. For example, a promyelocytic leukemia cell line, which consists of immature WBC, will have a greatly increased number of copies of an oncogene, which are overproducing the protein product. However, there are drugs which under some circumstances seem to be able to reverse the amplification process; with treatment, the cell loses the extra copies of the oncogene and becomes more like a mature normal WBC. One of these drugs, hexamethylbis (acetamide), is now entering clinical trials, and we eagerly await the correlation of clinical and molecular events. This area of cancer research, which deals with a redirection of a cancer cell back to normalcy, is gaining considerable impetus.

Summary

Insights from various areas of carcinogenesis can now be blended into cohesive and molecular hypotheses testable at a clinical level. One can now define new areas such as biochemical epidemiology. Whereas previously one thought of identifying individuals at high risk for cancer due to life-style or occupation, one can now propose to identify the susceptible individual at the molecular level for some cancers. Theoretically, if past exposure to carcinogens were significant, we may now be able to measure the exact sites of attack and damage in cellular DNA. We now have oncogenes as the probable targets. Treatment potential with highly specific molecular tools should not be far behind. As often cited before, the first priority of the NCI has always been basic research. The present excitement in the area of oncogenes has certainly been a shining example of research success.

As is always the case in a rapidly moving field, there are optimists and pessimists. There are fears of overpromise and dangers of lack of swift application. NCI's view can be summed up this way. Oncogene research is important if only for its implications in developmental biology. It needs no other reason for support or excitement. It also will be important to our understanding of cancer; how important, we do not yet know. We believe it will lead to practical applications in diagnosis, prevention, and treatment; how practical and how soon remain unknowns. By definition then, we are clearly optimists, for which no apologies are offered. The danger of overpromise, it seems to these authors, is exceeded by the risk of failure to pursue and apply one of the most exciting areas of research that brings molecular biology to the crowded bedside of the cancer patient. A good dose of optimism seems about right to make a little room.

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